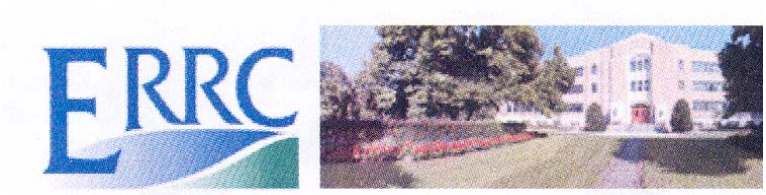

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Antioxidant Power, Lipid Oxidation, Color, and Viability of *Listeria monocytogenes* in Beef Bologna Treated with Gamma Radiation and Containing Various Levels of Glucose†

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ABSTRACT

Ionizing radiation can be used to pasteurize ready-to-eat (RTE) meat products. Thermal processing of RTE meats that contain dextrose results in the production of antioxidants that may interfere with ionizing radiation pasteurization of RTE meat products. Beef bologna was manufactured with dextrose concentrations of 0, 2, 4, 6, and 8%. Antioxidant activity, as measured by the Ferric Reducing Antioxidant Power assay, increased with dextrose concentration but was unaffected by ionizing radiation. Lipid oxidation increased significantly in irradiated bologna (4 kGy) that contained dextrose. Hunter color analysis indicated that the addition of dextrose reduced the ionizing radiation-induced loss of redness (a-value) but promoted the loss of brightness (L-value). The radiation resistance, D_{10}-value, of *Listeria monocytogenes* that was surface-inoculated onto bologna slices was not affected by dextrose concentration. *L. monocytogenes* strains isolated from RTE meats after listeriosis outbreaks were utilized. Increased antioxidant activity generated by thermal processing of dextrose in fine emulsion sausages does not present a barrier to radiation pasteurization of RTE meats. However, a high dextrose concentration in combination with gamma irradiation increases lipid oxidation significantly.

Listeria monocytogenes, a frequent postprocess contaminant in ready-to-eat (RTE) meat products, is a foodborne pathogen capable of growth at refrigerated temperatures and in high salt environments *(3, 5, 9, 27, 35)*. *L. monocytogenes* is given zero tolerance in RTE meat products in the United States because of the high mortality rate associated with listeriosis, which can be as high as 20%, in susceptible populations *(22, 42)*. Ionizing radiation can eliminate *L. monocytogenes* from raw, cooked, and cured RTE meat products *(32, 39, 41)*; however, the radiation resistance of *L. monocytogenes* can vary with product type *(36, 39)*.

A number of sweeteners, including dextrose, are commonly used in the manufacture of fine emulsion sausages such as bologna or frankfurters *(11, 28, 30)*. Both antioxidants and peroxides are produced by the thermal processing and radiolysis of dextrose, respectively *(6, 17, 20, 21, 24, 43)*. Maillard Reaction Products (MRPs) that form between dextrose and amino acids during cooking increase the antioxidant power of RTE meats *(6, 20, 21, 24)*. The addition of antioxidants into meats before irradiation can reduce changes in lipid oxidation, color, and off-flavor development as a result of irradiation *(2, 4, 10, 16, 19, 29)*. Both dextrose and MRPs inhibit *PrfA*-mediated virulence gene expression in *L. monocytogenes (7, 23, 34)*. Some authors have suggested increasing the carbohydrate concentrations of RTE meats or adding carbohydrate-derived antioxidants to RTE meats to inhibit virulence gene expression in *L. monocytogenes* during refrigerated storage *(23, 34)*.

Unfortunately, antioxidants can increase the radiation resistance of microorganisms *(18, 44)*. Antioxidants such as carnosine can increase the radiation resistance of *Aeromonas hydrophila* in meats *(40)*. Soy Protein Concentrate (SPC) can interfere with the elimination of *L. monocytogenes* from cooked beef bologna emulsion *(37)*. Sodium erythorbate, in solution, can increase the radiation resistance of *L. monocytogenes (38)*. Spices with antioxidant properties can protect *Escherichia coli* against the lethal effects of ionizing radiation *(33)*. The use of an additive to solve one problem has the potential to create another. The radiolysis of dextrose gives rise to peroxides *(17, 43)* that can negatively affect product antioxidant power and lipid oxidation but have the potential to increase the radiation sensitivity of *L. monocytogenes*.

What are the effects of these competing chemical processes on RTE meat antioxidant power, lipid oxidation, color, and survival of *L. monocytogenes?* To address these questions, beef bologna was manufactured with dextrose concentration as the only variable in the formulation. The effects of dextrose concentration and irradiation on bologna antioxidant activity, lipid oxidation, color, and radiation resistance of *L. monocytogenes* were determined.

MATERIALS AND METHODS

Bologna manufacture. Ground beef (15% fat) was emulsified in a Hobart Model HCM40 Cutter-Mixer. Cure ingredients and additives (wt/wt per kg meat) included 3% sodium chloride,

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† Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

TABLE 1. Listeria monocytogenes *strain information*[a,b]

Strain	Serotype	PFGE type	Source	D_{10} (±SE)	R^2
H7762	4b	e_1	Frankfurters	0.62 (±0.02)	0.98
H7596	Untypeable[c]	e_2	Deli turkey	0.46 (±0.02)	0.96
H7962	4b	e_0	Frankfurters	0.62 (±0.03)	0.95
H7969	4b	e_3	Frankfurters	0.60 (±0.02)	0.96
Scott A	ND	ND	Clinical isolate	0.48 (±0.05)	0.90

[a] PFGE, pulsed-field gel electrophoresis; ND, not determined.

[b] Strain source, serotype, and PFGE information were provided by the Centers for Disease Control and Prevention (Atlanta, Ga.). D_{10}-values are the mean of three independent experiments following surface inoculation onto beef frankfurters using the protocol of Sommers and Thayer *(39)*.

[c] Does not correlate with known serotypes.

0.5% sodium tripolyphosphate, 0.05% sodium erythorbate, 0.02% sodium nitrite, and 20% deionized water. Dextrose was added as needed to obtain the formulations required. Spices were not used in order to limit the number of experimental variables. The emulsion was stuffed into 4-in. (10-cm) fibrous casings (Dewied Int., Santa Fe, N.M.). The bologna was then cooked in a Koch Model KL-50 Smokehouse (Koch Inc., Kansas City, Mo.) to an internal product temperature of 73°C. The dry bulb setting was 90°C, and the wet bulb setting was 63°C, for a relative humidity of approximately 47%.

After the internal temperature was reached, the sausages were chilled using a sterile cold water bath. The sausages were then vacuum packaged to 0.26 mm Hg (1 mm Hg = 133.322 Pa) with a Multi-Vac A300 Vacuum Packager (Kansas City, Mo.), overpacked in gas- and moisture-impermeable Mil-B-131-H Foil Bags (Belle Fibre Products Corp., Columbus, Ga.), and stored at 0 to 2°C until ready for use. Immediately before individual experiments, the bologna was sliced to a thickness of 4 mm. Background microflora was monitored by pour plate assay (see below) over the course of the study and contributed less than 1 CFU/cm^2 surface area to the inoculated meat product at the lowest dilution used.

Strains. Four *L. monocytogenes* strains isolated from RTE meats (H7595, H7762, H7969, and H7962) were obtained from the Centers for Disease Control and Prevention (Atlanta, Ga.). The strains were propagated on Palcam Agar (Difco Laboratories, Detroit, Mich.) at 37°C and maintained at 0 to 2°C until ready for use. The identity of *Listeria* was confirmed by Gram stain, followed by analysis with Gram-Positive Identification cards using the Vitek Automicrobic System (bioMerieux Vitek, Inc., Hazelwood, Mo.). Radiation resistance, D_{10}-values, of the individual *L. monocytogenes* strains (Table 1) was determined using the protocol of Sommers and Thayer *(39)*. *L. monocytogenes* 49594 Scott A was obtained from the American Type Culture Collection (Manassas, Va.), and the D_{10}-value was determined for comparative purposes (Table 1).

Bacterial cultures. Each *L. monocytogenes* strain was cultured independently in 100 ml of tryptic soy broth (Difco) in baffled 500-ml Erlenmeyer culture flasks at 37°C (150 rpm) for 18 h. The cultures were then combined, and the mixture was sedimented by centrifugation (1,725 × *g* for 30 min). The *L. monocytogenes* cocktail was then concentrated 10-fold by resuspension in 40 ml of Butterfield's Phosphate Buffer (Applied Research Institute, Newtown, Conn.).

Inoculation. Single bologna slices were placed in no. 400 stomacher bags (Tekmar Co., Cincinnati, Ohio) and surface inoculated evenly onto one side with 0.2 ml of *L. monocytogenes* cocktail. The inoculated slices were then vacuum packaged to 0.26 mm Hg using a Multi-Vac Model A300 packager, overpacked in Mil-B Foil Bags (Belle), and stored at 0 to 2°C to await irradiation (approximately 30 min).

Gamma irradiation. A Lockheed Georgia Company (Marietta, Ga.) self-contained ^{137}Cs radiation source was used for all exposures. The radiation source consisted of 23 individually sealed source pencils placed in an annular array. The 22.9- by 63.5-cm cylindrical sample chamber was located central to the array when placed in the operating position.

The dose rate was 0.099 kGy/min. The temperature during irradiation was maintained at 4.0 ± 1.0°C by the gas phase of a liquid nitrogen source, which was introduced directly into the top of the sample chamber *(37, 39)*. The temperature was monitored by two thermocouples placed on the side of the sample bags. The dose delivered was verified by the use of 5-mm alanine pellet dosimeters, which were then measured by a Brucker EMS 104 EPR Analyzer. Radiation doses used were 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy for the dextrose portion of the study. To accommodate the wide range of D_{10}-values for individual *L. monocytogenes* strains, ionizing radiation doses of 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 kGy were used.

Plate counts. The samples were assayed for colony-forming units by standard pour plate procedures *(37, 39)*. For the surface-inoculated bologna slices, 100 ml of sterile Butterfield's Phosphate Buffer was added to a no. 400 stomacher bag that contained an inoculated slice, and the sample was mixed by shaking the contents approximately 50 times. The samples were then serially diluted in Butterfield's Phosphate Buffer, using 10-fold dilutions, and 1 ml of diluted sample was pour plated using *Listeria*-specific Palcam Agar (Difco). Three 1.0-ml aliquots were plated per dilution. The plates, typically containing 30 to 300 colonies, were then incubated for 48 h at 37°C before enumeration.

D_{10}-values. D_{10} is defined as the radiation dose required to effect a 90% reduction in viable organisms. The average colony-forming unit per square centimeter of an irradiated sample, at a specific dose (N), was divided by the average colony-forming unit per square centimeter of the untreated control (N_0) to produce a survivor ratio (N/N_0). D_{10} was determined by calculating the reciprocal of the slope provided by the $\log_{10}$ of the (N/N_0) ratios versus irradiation dose *(39)*.

Ferric Reducing Antioxidant Power assay. The antioxidant activity of cured meat was measured directly by the Ferric Reducing Antioxidant Power (FRAP) assay *(8)*. In the assay, the

antioxidants present reduce ferric tripyridyltriazine to the ferrous form, which has an intense blue color. Absorbance was measured at 593 nm, and concentration was calculated against a standard curve of ascorbic acid (0 to 500 μM). Bologna slices were vacuum packaged (n = 6) in no. 400 stomacher bags and irradiated as previously described to doses of 0, 2.0, and 4.0 kGy. After irradiation, the samples were stored at −70°C until analyzed. One hundred milliliters of sterile distilled water was then added to the slice, the sample was macerated and mixed by stomaching for 90 s, and 0.1 ml of the aqueous phase was used for FRAP value determination. FRAP values were expressed as micromoles per gram of bologna.

Lipid oxidation. Lipid oxidation was measured using the thiobarbituric acid (TBA) assay modified from the methods of Hodges et al. *(13)* and Zipser and Watts *(45)*. Ten grams of bologna was homogenized with 25 ml of 0.5 M phosphate (pH 2.5) buffer containing 0.08% sulfanilamide and 0.01% butylated hydroxytoluene using a homogenizer (Virtishear, Virtis, Gardiner, N.Y.) at a speed setting of 70 for 1 min. The homogenate was filtered through a Whatman #2 paper filter (Whatman, Inc., Clifton, N.J.), and then the filtrate was centrifuged at 1,300 × g for 10 min at 5°C in a Sorvall RT6000B refrigerated centrifuge (DuPont Co., Wilmington, Del.). A 1.6-ml aliquot of the supernatant was added to a test tube containing 1.6 ml of either (i) −TBA solution: 20% (wt/vol) trichloroacetic acid and 0.01% butylated hydroxytoluene, or (ii) +TBA solution: containing the above plus 0.65% TBA. Samples were then mixed vigorously, heated at 95°C in a water bath for 25 min, and cooled and centrifuged at 1,300 × g for 10 min at 5°C. Absorbance at 440, 532, and 600 nm was monitored by a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, Md.). TBA-reactive substance (TBARS) values were expressed as the malondialdehyde (MDA) equivalent and calculated using the formulas developed by Hodges et al. *(8)*.

$$[(\text{Abs}532_{+\text{TBA}} - \text{Abs}600_{+\text{TBA}}) - (\text{Abs}532_{-\text{TBA}} - \text{Abs}600_{-\text{TBA}})] = A \quad (1)$$

$$[(\text{Abs}440_{+\text{TBA}} - \text{Abs}600_{+\text{TBA}})0.0571] = B \quad (2)$$

$$\text{MDA (nmol/g)} = [(A - B)/157{,}000]10^6 \quad (3)$$

Color analysis. Bologna slices were packed and irradiated as described previously *(37)*. Color analysis was then performed using a Hunter Lab Miniscan XE Meter (Hunter Laboratory, Inc., Reston, Va.). The meter was calibrated using white and black standard tiles. Illuminant D65, 10° standard observer, and a 2.5-cm port/viewing area were used. Six readings were taken per parameter.

Statistical analysis. Statistical analysis was completed using the statistical analysis package of Microsoft Excel (Redmond, Wash.) and SAS Version 6.12 (SAS Institute, Cary, N.C.). A comparison of regressions (D_{10}-values) was performed using analysis of covariance (ANCOVA) *(39)*. Sigma Plot Version 5.0 (SPSS, Inc., Chicago, Ill.) was used for graphic presentation of the data.

RESULTS

The antioxidant activity of unirradiated and irradiated (2 and 4 kGy) bologna slices containing variable concentrations of dextrose (0, 2, 4, 6, and 8%) was determined (Fig. 1). FRAP values of unirradiated bologna increased significantly as a function of dextrose concentration as determined by analysis of variance (ANOVA) (n = 3, α = 0.05) (Fig. 1). FRAP values of unirradiated bologna were 978 (±161), 1,229 (±342), 1,356 (±310), 1,614 (±231), and 1,683 (±500) μmol/g in bologna emulsion that contained 0, 2, 4, 6, and 8% dextrose, respectively. The FRAP values obtained from bologna irradiated to 2 and 4 kGy also increased significantly as a function of dextrose concentration ($P < 0.05$) (Fig. 1). However, when FRAP values were compared as a function of gamma radiation dose, no statistically significant radiation dose-dependent increase was evident as determined by ANOVA (n = 3, α = 0.05). Dextrose concentration, but not gamma radiation, affected the antioxidant power of the bologna.

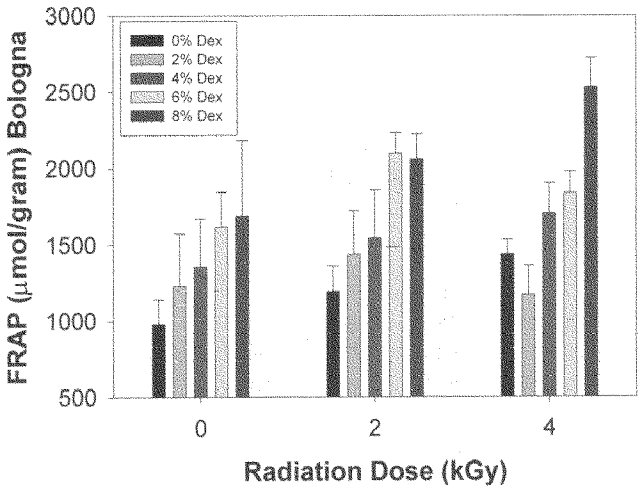

FIGURE 1. *Soluble antioxidant power of cooked beef bologna emulsion containing various concentrations of dextrose as determined by the Ferric Reducing Antioxidant Power (FRAP) assay. FRAP values are expressed as micromoles of antioxidant per gram. Vacuum-packaged bologna was either unirradiated or irradiated to 2.0 or 4.0 kGy. Each experiment was conducted independently three times. Standard errors are shown for each data set.*

Lipid oxidation, as measured by the TBARS assay, increased linearly as radiation dose increased ($P < 0.01$), and the increase was observed at all dextrose concentrations (Fig. 2). The TBARS values of bologna irradiated to 4 kGy increased linearly ($P < 0.01$) as dextrose concentration increased, whereas those irradiated at other doses (0 and 2 kGy) did not have significant changes in TBARS with dextrose concentration.

Hunter color analysis of unirradiated and irradiated bologna slices revealed significant changes in product color as a function of dextrose concentration and radiation dose (Fig. 3). Redness (a-value) increased significantly ($P < 0.05$) in unirradiated bologna slices that contained 4, 6, and 8% dextrose as determined by ANOVA (n = 6, α = 0.05). Redness decreased significantly as a result of irradiation at all dextrose concentrations tested as determined by ANOVA (n = 6, α = 0.05). However, in bologna containing 6 and 8% dextrose, the a-value decreased to the same level as that observed in unirradiated bologna that contained no dextrose. Yellowness (b-value) was unaffected by either dextrose concentration or radiation dose as determined by ANOVA (n = 6, α = 0.05) (Fig. 3). Brightness, L-values (Fig. 3), decreased as a function of dextrose concentration

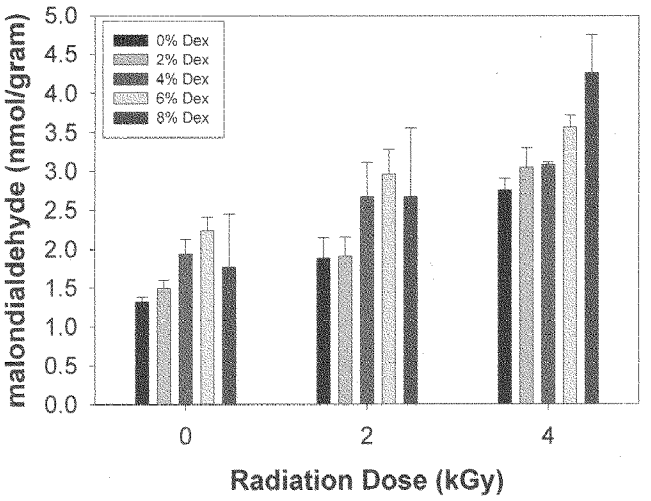

FIGURE 2. *Lipid oxidation in vacuum-packaged unirradiated and irradiated (2.0 and 4.0 kGy) bologna that contained various dextrose concentrations as determined by the Thiobarbituric Acid-Reactive Substances (TBARS) assay. Results are expressed as nanomoles per gram of malondialdehyde (MDA) formed. Each experiment was conducted independently three times. Standard errors are shown for each data set.*

but not as a function of ionizing radiation dose as determined by ANOVA ($n = 6$, $\alpha = 0.05$).

L. monocytogenes strains, obtained from the Centers for Disease Control and Prevention, that were associated with foodborne illness outbreaks because of the consumption of contaminated RTE meats were used in this study. Strain information, including the first reporting of the D_{10}-values for those strains, is included in Table 1. D_{10}-values for the *L. monocytogenes* mixture were 0.60 (±0.04), 0.60 (±0.03), 0.59 (±0.02), 0.60 (0.02), and 0.61 (0.04) kGy on bologna slices that contained 0, 2, 4, 6, and 8% dextrose, respectively (Fig. 4). Despite the dextrose-dependent increase in antioxidant activity, there was no significant difference in D_{10}-values for *L. monocytogenes* surface inoculated onto the bologna as determined by ANCOVA ($n = 3$, $\alpha = 0.05$).

DISCUSSION

A petition has been filed with the U.S. Food and Drug Administration to allow ionizing radiation pasteurization of RTE meat products (National Food Processors Association, 1999). The primary function of ionizing radiation pasteurization is to eliminate harmful microorganisms, including *L. monocytogenes*. Sommers and Thayer *(39)* found that the radiation D_{10}-values of *L. monocytogenes* inoculated onto commercially available frankfurters ranged from 0.49 to 0.71 kGy, with a mean value of 0.61 kGy, and speculated that product formulation and surface treatments might be responsible for the differences. The D_{10}-values of the strains isolated from RTE meats (Table 1) and used as a mixture to inoculate bologna ranged from 0.46 to 0.62 kGy. The strain(s) selected, in addition to product formulation, can affect the ionizing radiation dose required to reduce an *L. monocytogenes* population by 5 $\log_{10}$ in viable cell counts, which would range from 2.3 to 3.1 kGy for those used in this study. The use of a single strain might have resulted in an underestimation of D_{10}.

Dextrose is a common sweetener used in the manufacture of RTE meats, and the concentration used in RTE meats can vary considerably *(28, 30)*. The thermal processing of dextrose in the presence of amino acids results in the production of antioxidants that could positively affect product quality but increase the radiation resistance of *L. monocytogenes (6, 20, 21, 24)*. In contrast, gamma irradiation of dextrose leads to the production of peroxides that could negatively affect RTE meat product quality *(17, 43)*. We determined the effect of dextrose, in combination with ionizing radiation, on the radiation resistance of *L. monocytogenes*, lipid oxidation, antioxidant activity, and color of beef bologna.

Antioxidants have the potential to protect microorgan-

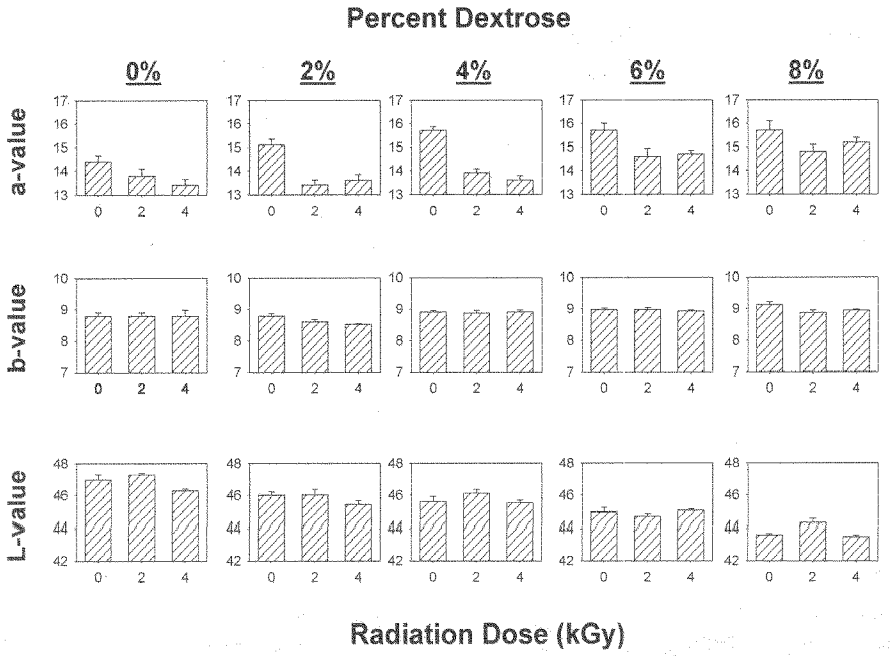

FIGURE 3. *Color of vacuum-packaged unirradiated and irradiated (2.0 and 4.0 kGy) beef bologna slices that contained various dextrose concentrations as determined by Hunter color analysis. Redness (a-value), yellowness (b-value), and brightness (L-value) are shown for each experimental condition. Each experiment was conducted independently six times. Standard errors are shown for each data set.*

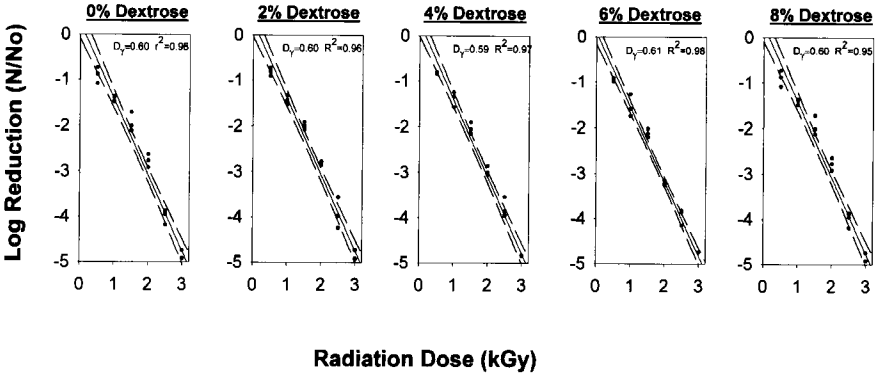

FIGURE 4. *Radiation resistances (D_{10}-values) for a four-strain* L. monocytogenes *cocktail that was surface inoculated onto beef bologna slices that contained various dextrose concentrations. Gamma radiation doses were 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy. The dose rate was 0.099 kGy/min. Each experiment was conducted independently three times. Individual log_{10} reduction points are shown as closed circles, regressions as solid lines, and 95% confidence limits as dashed lines.* L. monocytogenes *D_{10}-values and R^2 values are listed for each dextrose concentration.*

isms from the lethal effect of ionizing radiation by scavenging the radiolysis products of water and other compounds produced by exposure to ionizing radiation *(33, 37, 38, 40, 44)*. The antioxidant activity of cooked bologna emulsion increased as a function of dextrose concentration. The FRAP values obtained in these experiments ranged from a low of 978 μmol/g in unirradiated bologna that contained no dextrose to almost 2,500 μmol/g in 8% dextrose bologna irradiated to 4 kGy. The increased dextrose-dependent antioxidant activity had no effect on the radiation D_{10}-values of *L. monocytogenes*. In contrast, antioxidants in SPC were found to increase the radiation resistance of *L. monocytogenes* in a previous study. Sommers et al. *(37)* found that *L. monocytogenes* was slightly more resistant to ionizing radiation when inoculated onto bologna emulsion that contained 3.5% SPC (5,994 μmol/g FRAP) as opposed to bologna emulsion that contained 1.75% SPC (3,572 μmol/g FRAP) or no SPC (1,958 μmol/g FRAP). While it is sometimes difficult to compare interexperimental results, the antioxidant activities obtained in these experiments were considerably less than the 5,994 μmol/g FRAP that increased the radiation resistance of *L. monocytogenes* in bologna containing SPC. Also, the possibility exists that thermally derived antioxidants produced from dextrose may have reacted with dextrose-derived peroxides, nullifying any potential protection afforded to *L. monocytogenes* against the lethal effects of ionizing radiation.

The TBA assay, measuring the quantity of MDA, is widely used for assessing the oxidative rancidity of meats and other fat-containing food products. The assay is, however, not specific. Many compounds, such as aldehydes, nitrite, and sugars, may interfere with the assay *(12, 31)*. The method used in the present study was developed specifically for foods containing carbohydrates and nitrite. Ionizing radiation-induced increases in TBARS values have been observed in earlier studies *(10, 15, 16, 37)*, and the formation of TBARS because of irradiation is more pronounced in aerobically packed meats than in vacuum-packed meats *(1, 25, 26)*. Our results suggest that irradiation increased the lipid oxidation in cooked vacuum-packed bologna. Irradiation accelerates the oxidative breakdown of polyunsaturated fatty acids and consequent MDA formation. MDA may also be generated from carbohydrates. It has been shown that irradiation increases TBARS values of carbohydrate-rich foods and aqueous carbohydrates *(17, 31)*.

The linear relationship between TBARS values and dextrose concentration observed in the bologna irradiated to 4 kGy is probably because of the formation of MDA and perhaps other TBA-reactive compounds from dextrose during irradiation. Although MRPs formed from sugars and amino acids have been shown to possess antioxidant activity and may potentially decrease TBARS values in bologna, radiation-induced MDA formation from dextrose may outweigh the antioxidant activity of MRPs, resulting in higher overall TBARS values.

Ionizing radiation-induced loss of redness in cured meat products has been observed in a number of studies *(10, 14, 25, 26, 37)*. Color analysis of unirradiated and irradiated bologna slices indicated that dextrose concentrations greater than 4% can decrease the loss of radiation-induced redness in beef bologna. However, the higher dextrose concentrations also induced a clearly visible loss of product brightness in the beef bologna slices examined. It should be noted that the higher concentrations of dextrose are well above the median values for sugars in RTE meats found by Sommers and Thayer *(39)* and could be considered an unwelcome addition to the diet. The effect of high dextrose concentration and ionizing radiation on lipid oxidation in beef bologna indicates that high dextrose concentrations or MRP supplementation of RTE meats would not be a practical method for color preservation or for *L. monocytogenes* virulence factor inhibition of those products.

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